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VITAMIN A DEFICIENCY MODIFIES RESPONSE OF PREDATORY MITE *Amblyseius potentillae* TO VOLATILE KAIROMONE OF TWO-SPOTTED SPIDER MITE, *Tetranychus urticae*

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Abstract—A volatile kairomone of the two-spotted spider mite, *Tetranychus urticae*, elicits a searching response of the phytoseiid predator *Amblyseius potentillae*, only when the predator is reared on a carotenoid-free diet. However, after addition of crystalline β-carotene or vitamin A acetate to the predator's rearing diet this searching response was absent. Because vitamin A and β-carotene are indispensable nutrients for diapause induction, the carotenoid-deficient predators increase their fitness by searching for two-spotted spider mites, when other spider mites are unavailable. Two-spotted spider mites, among others, contain the carotenoids required for diapause induction, but are an inferior prey due to the dense webbing they produce. When the predators have carotenoids at their disposal, they do better by searching for other spider mites that are more profitable in terms of reproductive success. Such a prey is the European red spide mite, *Panonychus ulmi*. The volatile kairomone of this prey elicits a searching response of the predator whether it has a carotenoid deficiency or not.

Key Words—*Amblyseius potentillae*, *Tetranychus urticae*, *Panonychus ulmi*, Acarina, Phytoseiidae, Tetranychidae, volatile kairomones, vitamin A, β-carotene, diapause induction.

INTRODUCTION

Amblyseius potentillae (Garman) (Acarina: Phytoseiidae) is a polyphagous predatory mite that can feed and reproduce on phytophagous spider mites (Acar-

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ina: Tetranychidae) (Kropczynska-Linkiewicz, 1971; Overmeer, 1981) and pollen of several plant species (Overmeer, 1981). The predator is known to respond to volatile kairomones. In Y-tube olfactometer experiments it was shown that starved *A. potentillae,* when reared on two-spotted spider mites (*Tetranychus urticae* Koch), respond to a volatile kairomone of the European red spider mite [*Panonychus ulmi* (Koch)] but not to a kairomone emitted from leaves infested by the two-spotted spider mite, to which other predatory mites respond (Sabelis and Van de Baan, 1983). *Tetranychus urticae* is an unprofitable prey species for *A. potentillae*, as the predator is hindered by the dense webbing produced by this spider mite (Sabelis, 1981). The European red spider mite that also produces silk but not a dense webbing is a more profitable prey. These differences in values of these prey species for *A. potentillae* also are reflected in the ability of the predator to control populations of these mite species. The predator is able to control populations of *P. ulmi* Van de Vrie, 1973; McMurtry and Van de Vrie, 1973; Rabbinge, 1976), but it is unable to control those of *T. urticae* (Sabeis, 1981).

When animals search for food, they have to make decisions about where to search, how long to search at a specific site, and which type of food to eat. In optimal foraging theory, it is assumed that predators make decisions so as to maximize their genetic contributions to future generations. It is therefore assumed that animals maximize their net rate of food intake (Pyke et al., 1977; Krebs, 1978). Although it has received little emphasis in the literature, nutrient composition also is important and should be optimized. Examples are available for spiders (Greenstone, 1979) and moose (Belovsky, 1978). General dietary components like nitrogen, amino acids, and proteins are usually thought to be important (Greenstone, 1979; Slansky and Feeny, 1977; Moss et al., 1972). In this paper, a specific nutrient is taken into account: vitamin A, which is an indispensable nutrient for diapause induction in the eyeless mite *Amblyseius potentillae* (Veerman et al., 1983). Our work shows that a deficiency of vitamin A or its precursor β-carotene in the diet of *A. potentillae* affects its response to a kairomone of one of its prey species, the two-spotted spider mite. To our knowledge this is the first time that any single nutrient has been shown to be of crucial importance in prey selection.

METHODS AND MATERIALS

Predators. Amblyseius potentillae was reared on plastic plates (McMurtry and Scriven, 1965) in a climate room at 25 $\pm$ 1°C, 60 $\pm$ 10% relative humidity, under continuous fluorescent light on four different diets. Two-spotted spider mites have been used as prey for many years. The mites were brushed off the host-plant leaves onto the plastic plates to circumvent the adverse effects of the dense webbing of this prey. For about five years, predators from this culture

have been reared on broad bean (*Vicia faba* L.) pollen, a carotenoid-free diet for *A. potentillae* (Overmeer and Van Zon, 1983). For some experiments we added crystalline β-carotene or vitamin A acetate (Sigma Co., St. Louis, Missouri) to the pollen (1 mg β-carotene or vitamin A acetate per 5 mg pollen). Overmeer and Van Zon (1983) and Veerman et al. (1983) showed that *A potentillae* take up these crystalline carotenoids when mixed with pollen.

Prey. The two-spotted spider mite was reared in our laboratory for many years on Lima beans (*Phaseolus lunatus* L.) at 20–30°C under continuous fluorescent light that was added to the sunlight regime.

The European red spider mites were collected from an apple orchard. The leaves, on which 20–40 active *P. ulmi* stages were present, were used in the bioassay on the day collected.

Bioassay. We used an olfactometer that consisted of a glass tube with an iron wire in the center, both of which are Y-shaped. Odor-emitting objects may be placed in PVC cages at the ends of both arms of the Y-tube olfactometer. Air was sucked out at the base of the tube and led to the outside of the climate room in which the olfactometer was positioned. The airstream speeds in both arms of the olfactometer were measured with a hot wire anemometer and standardized at 0.7 ± 0.1 m/sec by inserting dry cotton wool in the inlet of the airstream and/or changing the total air speed with a valve. Female predators that had been deprived of food and water for 20 hr in a plastic tube were placed individually on the wire at the base of the basal tube and observed. Starved predators were used as these showed a response to the volatile kairomone of *P. ulmi*, unlike well-fed predators (Sabelis and Van de Baan, 1983). When the predator walked upwind and reached the far end of one of the arms, the experiment was terminated. The maximum experimental time was 10 min. Predators that did not walk to the far end of one of the arms were left out of the statistical analysis.

The results have been analyzed using a sign test. The null hypothesis is that the probabilities of the predators to walk to the far end of either arm are equal to 50%. For a more detailed description of the olfactometer, the experimental procedure and statistical analysis, see Sabelis and Van de Baan (1983). The experiments were performed at 26 ± 1°C and 60 ± 10% relative humidity.

RESULTS AND DISCUSSION

Results of experiments using the Y-tube olfactometer show that when *A. potentillae* were reared on broad bean pollen and then starved for 20 hr, they responded to the volatile kairomones of *T. urticae* and *P. ulmi*. However, when they were reared on *T. urticae*, they did not respond to the kairomone of *T. urticae*, but only to that of *P. ulmi* (Table 1). The difference in response between predators reared on the two different diets could be accounted for either

TABLE 1. RESPONSE OF STARVED FEMALE *A. potentillae* TO KAIROMONES OF DIFFERENT SPIDER MITE SPECIES IN Y-TUBE OLFACTOMETER

Predator reared on	Content of arm 1	Content of arm 2	N^a	$N(1)$	$N(2)$	$N(0)$	Critical level
T. urticae	9 bean leaves infested with *T. urticae*	9 clean bean leaves	40	21	19	0	NS[b]
	18 apple leaves infested with *P. ulmi*	18 clean apple leaves	40	31	9	0	$P < 0.001$[b]
V. faba pollen	9 bean leaves infested with *T. urticae*	9 clean bean leaves	60	44	15	1	$P < 0.001$
	18 apple leaves infested with *P. ulmi*	18 clean apple leaves	53	38	14	1	$P < 0.001$

[a] N = number of predators tested; $N(1)$ = number of predators that walked to far end of arm 1; $N(2)$ = number of predators that walked to far end of arm 2; $N(0)$ = number of predators that did not walk to far end of one of the arms.
[b] Results of Sabelis and Van de Baan (1983).

by a genetic difference due to different selection pressures in the two cultures or by a physiological difference due to different diets. No differences in fecundity, developmental time, and mortality have been observed between *A. potentillae* reared on *T. urticae* or *V. faba* pollen (Overmeer, 1981). The predators can be reared equally well on both diets. However, it has been reported that predators reared on broad bean pollen cannot enter reproductive diapause, while those reared on *T. urticae* can (Overmeer and Van Zon, 1983). Because β-carotene is known to affect diapause in the two-spotted spider mite (Veerman and Helle, 1978), this compound was added to the broad bean pollen or to carotenoid-free eggs of albino *T. urticae* that *A. potentillae* were reared upon. This addition restored the predator's ability to enter diapause (Van Zon et al., 1981; Overmeer and Van Zon, 1983). Adding vitamin A, a derivative of β-carotene, to the diet of *A. potentiallae* also has the same effect (Veerman et al., 1983). Thus, a physiological difference between predators reared on the two different diets might cause the difference in response to the *T. urticae* kairomone.

When *A. potentillae* were reared for several generations on broad bean pollen mixed with β-carotene or vitamin A or just on broad bean pollen to which the carotenoid was only added five days prior to the experiment, the predators showed no response to the kairomone of *T. urticae* (Table 2). However, the response to the kairomone of *P. ulmi* was still present when the predators were reared for several generations on broad bean pollen to which crystalline β-carotene was added. Thus, the response of *A. potentillae* to the kairomones of *T. urticae* and *P. ulmi* is identical for predators reared on either *T. urticae* or pollen plus crystalline β-carotene as carotenoid source. These results show that β-

TABLE 2. RESPONSE IN Y-TUBE OLFACTOMETER OF STARVED FEMALE *A. potentillae* REARED ON DIET OF *V. faba* POLLEN TO WHICH CRYSTALLINE CAROTENOIDS WERE ADDED

Predators reared on	Content of arm 1	Content of arm 2	N^a	$N(1)$	$N(2)$	Critical level
V. faba pollen + β-carotene	9 bean leaves infested with *T. urticae*	9 clean bean leaves	40	20	20	NS
V. faba pollen (β-carotene was added 5 days prior to the experiment)	9 bean leaves infested with *T. urticae*	9 clean bean leaves	41	17	24	NS
V. faba pollen + vitamin A acetate	9 bean leaves infested with *T. urticae*	9 clean bean leaves	42	19	23	NS
V. faba pollen + β-carotene	18 apple leaves infested with *P. ulmi*	18 clean apple leaves	40	30	10	$P < 0.001$

[a] N = number of predators tested; $N(1)$ = number of predators that walked to far end of arm 1; $N(2)$ = number of predators that walked to far end of arm 2.

carotene and vitamin A affect the response to the *T. urticae* kairomone. Predators respond to this kairomone when they lack these carotenoids. Because vitamin A is an essential nutrient for inducing diapause and hence for hibernation, it is thus worth foraging for, since the ability to enter diapause is of selective advantage. Predators being under stress conditions (in this case carotenoid-deficiency) would do better by broadening their diet, and the response to the *T. urticae* kairomone by carotenoid-deficient predators can be interpreted in this context.

Amblyseius potentillae is found in orchards, where it is unlikely that the pollen of *V. faba* would be an important food source. Therefore one wonders under what circumstances carotenoid shortage could occur in the field. This question cannot be answered yet, but is of importance in concluding how frequently carotenoids are a limiting factor, and thus how decisive their role is in natural selection.

There are several ways in which *A. potentillae* can obtain carotenoids, and it would be interesting to study how they are affected by depriving *A. potentillae* of carotenoids: (1) Predation on any seizable phytophagous prey. When deprived of carotenoids *A. potentillae* responds to the kairomones of more prey species than after consumption of these compounds (Dicke and Groeneveld, 1986; this paper). (2) Cannibalism, a phenomenon often observed in cultures of *A. poten-*

tillae on broad bean pollen, but not in those on *T. urticae* (Overmeer and Van Zon, 1983; own observation). This only helps the carotenoid lack when the conspecific contains cartenoids. (3) Consumption of the types of pollent that have carotenoids available to the predator. (4) Consumption of red yeasts that are present on the host plant leaf. The phytoseiid mite *Amblyseius finlandicus* (Oudemans) has been found to feed and reproduce on fungal spores (Kropczynska, 1970), but so far *A. potentillae* has not been found to feed on fungi. (5) Phytophagy, a phenomenon reported for the predatory mite *A. hibisci* (Chant) (Porres et al., 1976) that has recently been confirmed for *A. potentillae* as well (Dicke, in preparation).

Thus, it might seem that, in the field, *A. potentillae* usually should have no problem in obtaining carotenoids. Whether this is true should be investigated by sampling predators in the field and testing their ability to enter diapause. However, if obtaining carotenoids is indeed not a problem for *A. potentillae*, this might be the result of the development in evolutionary time of one or more of the five above-mentioned strategies to obtain carotenoids. As carotenoids are unstable under light conditions, this might mean that the transparent *A. potentillae* has to replenish its supply regularly. Still, the amount of carotenoids needed for diapause induction seems to be small; after switching from a diet with carotenoids to one without, the offspring do not loose the ability to enter diapause until in the second generation (Overmeer and Van Zon, 1983). However, a low amount of carotenoids may be insufficient to detect the photoperiodic signal under dim light conditions, as has been reported for larvae of *Bombyx mori* (Shimizu and Kato, 1984).

Whether other functions of carotenoids in *A. potentillae* exist is not known. In other organisms, only photofunctions have been demonstrated for carotenoids (Krinsky, 1971), although many hypotheses about other functions have been put forward (Krinsky, 1971; Karnaukhov et al., 1977). Based on their isolation from bovine olfactory epithelium and the brownish-yellow color of olfactory mucosa, it has also been suggested that carotenoids function as receptors for the energy from odorous molecules (Briggs and Duncan, 1961; Kurihara, 1967). It was thus postulated that smell is impaired by vitamin A deficiency (Briggs and Duncan, 1961). In *A. potentillae*, vitamin A deficiency results in response to the kairomones of an increased number of prey species. In Y-tube olfactometer experiments in which different prey species were offered in each olfactometer arm, carotenoid-deficient *A. potentillae* distinguished between the kairomones of these prey species (Dicke and Groeneveld, 1986). This indicates that olfactory discrimination in *A. potentillae* is improved. The hypothesis of Briggs and Duncan (1961) would predict, however, an impaired olfactory function, and thus this hypothesis cannot hold for olfaction in *A. potentillae*.

A response to kairomones of a larger number of prey species can be thought to occur under stress situations other than carotenoid deficiency. Severe star-

vation can be such a stress situation. A response to the volatile kairomone of *T. urticae* may therefore be present in *T. urticae*-reared *A. potentillae* after starvation for a longer time than the 20 hr of food deprivation used in this study.

Conditioning due to experience with a host species has been observed to affect host recognition by hymenopterous parasitoids (Arthur, 1971; Vinson et al., 1977; Vet, 1983). In the present case, predators that were reared on two-spotted spider mites for many years do not respond to the volatile kairomone of this prey species (Sabelis and Van de Baan, 1983). Only predators that were reared on broad bean pollen and never had any contact with two-spotted spider mites respond to the volatile kairomone of this prey. The response of *A. potentillae* to the volatile kairomone of *T. urticae* is not dependent on previous contact with this prey species, but on whether the predator has available nutrients that are indispensable for diapause induction. The eyeless predatory mite *A. potentillae* can identify a deficiency in β-carotene or vitamin A in its body. As a result, the predator responds to a volatile kairomone of an unprofitable prey species when the alternative is no prey at all. The result of this response is that after finding and consuming this prey species, the predator will have carotenoids available and thus will be able to enter diapause.

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